REMARKS

Claims 27, 28, 36, and 40 have been amended to specify that the spacer comprises a double-stranded DNA sequence of at least 50 nucleotides and wherein said sequence is not present in the cell, and that the kit also comprises a second labeled antibody directed against the primary antibody or the specific hypervariable portion thereof, wherein said second labeled antibody is conjugated with an enzyme. Support for the amendments can be found in the Specification as filed, for example, in claims 28, 32, 39, 40, 41, paragraphs [0028], [0077], and [0089]. For example, in paragraph [0077] (Example 1), a double-stranded spacer sequence from CMV (i.e. a viral sequence) is linked to probes containing binding sites for NFkB, CREB, and AP-1 (i.e. cellular transcription factors). Therefore, the spacer sequence is not present in the biological sample that naturally has the NFkB, CREB, and AP-1 transcription factors.

Claims 31, 32, 39 and 41 have been cancelled. New Claims 42 and 43 have been added. Support for the new claims can be found in the Specification as filed, for example, in paragraphs [0034], [0050] and [0109]. No new matter has been introduced by these amendments. The following addresses the substance of the Office Action.

Definiteness

The Examiner has rejected claim 40 under 35 USC §112, second paragraph, as being indefinite. Specifically, Claim 40 is asserted to be unclear for reciting the limitation "such as". Such limitation is also part of claim 28. Applicant has amended Claims 28 and 40 by canceling "an enzyme such as".

Therefore, Applicants respectfully request that the rejection of Claims 28 and 40 under 35 USC §112, second paragraph as indefinite be withdrawn.

Non-obviousness

The Examiner has rejected Claims 27-29, 31-33 and 36-38 under 35 USC §103(a) as being allegedly unpatentable over Peterson et al. (US 5,563,036) in view of Hibma et al. (1994 *Nucl. Acids Res.* **22**:3806-3807), and further in view of Kaltschmidt et al. (*Biol. Chem. Hoppe-Seyler* **376**: 9-16; 1995). Specifically, the Examiner stated that a person of ordinary skill in the art would have been motivated to combine the product element of Peterson et al. with the labeled antibodies of Hibma et al. wherein the antibody is specific to the activated form of NF-kB, as described by Kaltschmidt et al.

> To establish a *prima facie* case of obviousness a three-prong test must be met. First, there must be some suggestion or motivation, either in the references or in the knowledge generally available among those of ordinary skill in the art, to modify the reference. Second, there must be a reasonable expectation of success found in the prior art. Third, the prior art must reference must teach or suggest all the claim limitations. *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991).

As discussed in the Inventor's Declaration filed herewith under 37 C.F.R §1.132, the inventors developed a mechanism for assessing the activation state of transcriptional factor(s) present in a cell or cell lysates or for the screening and/or quantification of (a) compound(s) able to bind to said activated transcriptional factor(s) or inhibit the binding of said activated transcriptional factor(s) to a specific nucleotide sequence (see paragraphs 5-7). The inventors tested the effects of the presence or absence of a spacer separating the specific sequence for transcription(al) factor(s) binding from the solid support, as well as the length and the type of the spacer, on the final combination of high specificity and high sensitivity of the assay and kit. The inventors showed that when activated transcription factors are contacted with short double-stranded DNA sequences without a spacer, the assay is quite specific but not sensitive at all (i.e. giving false negative results). When activated transcription factors are contacted with long double-stranded DNA sequences without a spacer, the assay is sensitive but not specific (i.e. giving false positive results).

However, Applicants unexpectedly discovered that activated transcription factors present at very low amounts in a cell or cell lysate can be detected in a binding assay combining double-stranded DNA sequences comprising the binding sites for the transcription factors and a spacer comprising at least a double-stranded nucleotide sequence of at least 50 nucleotides where the nucleotide sequence of the spacer is not present in the tested cell (see paragraph 8). When such constrained specifications are met, the assay becomes both sensitive enough to assay biological samples and specific (suppressing both the false negative and false positive results) even when a plurality of transcription factors are simultaneously assayed on a micro-array. Furthermore, the kinetics of binding is sufficient to permit obtaining results in a very short time frame.

Thus, the data presented in the Declaration unambiguously show that the kit as claimed is sensitive and specific (see paragraph 9).

The high sensitivity obtained with the present kit allows the use of non radioactive detection means, and provides advantages over the method of Peterson et al.: a non radioactive

detection method avoids the drawbacks linked to the use of radioactive labels; quantification of the activated transcription factors is possible because the signal obtained reflects the binding of the activated transcription factors to their specific binding sequence only, and not to the spacer nucleotide sequence; and the kit allows the detection of very small amounts of activated transcription factors among paramount amounts of other proteins, which is a hallmark of cell-derived samples.

Peterson et al. describe double-stranded DNA sequences having at least a portion of nucleotide sequence naturally involved in the regulation of the transcription of the gene which is necessary for sequence-specific interaction with the transcription factor. The nucleic acid may be preferably between about 18 bp and 250 bp. The Examiner states that the nucleic acid contains at least a portion of which is common to the gene regulatory region to which the transcription factor normally binds, the binding site portion constituting between 4 and 8 nucleotides, and that the remaining sequence length would constitute a spacer sequence (column 6, lines 35-38). It is understood from the reading of this sentence that the remaining sequence is a sequence adjacent to the portion of gene or gene regulatory region to which the native transcription factor binds, thus the remaining sequence is part of the gene or gene regulatory region.

However, a nucleotide sequence of 18 to 250 bp naturally involved in the regulation of the transcription of the gene, as taught by Peterson, cannot be used to reach specific transcription factor detection. To illustrate this, the sequence of 46 bp given in the example from Peterson et al., designed to bind NFkB (col. 13, lines 10-12), was analyzed using the TFSEARCH engine (on WorldWide Web at cbrc.jp/research/db/TFSEARCH.html; limitation to the vertebrate matrix) (see paragraph 12). As expected, NFkB specific sites were identified (two sites, corresponding respectively to base pairs 10-19 and 31-40). However, binding sites for other factors were found to overlap these sites: NFkB site 1 is overlapped by sites for MZF1, GATA-3 and GATA-1, while NFkB site 2 is overlapped by sites for ADR1 and Ik-2.

Furthermore, as provided by the literature, non protected regulatory regions of a gene may be the targets of numerous transcription factors which may interfere with the assay (see, Yang et al. (2007) *Annals of Biomedical Engineering*, 35(6): 1053-1067, attached).

Peterson et al. neither suggest nor mention the use of a spacer comprising a double-stranded nucleotide sequence which is at least 50 nucleotides long and which is not found in the cell containing the activated transcription factors to assay.

Furthermore, there is no teaching or suggestion in Peterson et al. of a kit comprising a second labeled antibody directed against the primary antibody or the specific hypervariable portion thereof, wherein said second labeled antibody is conjugated with an enzyme.

Hibma et al. describe an ELISA-based assay for detecting the binding of a transcription factor to its cognate DNA sequence using antibodies directed to the transcription factor. In Hibma et al., the protein to be detected, as illustrated in the example with papillomavirus type 16 E2 protein, is a recombinant protein not naturally present in the cell. The transcription factors which reside in cells are numerous and present at very low concentration. The activated transcription factor molecules, which are typically a small subfraction of the total transcription factor molecules, are present at even lower concentrations.

It was not obvious to detect such low concentrations of activated transcription factors by applying the enzymatic detection of Hibma et al. The solution is provided by the instant claim 27, through the use of a spacer comprising a double-stranded nucleotide sequence of at least 50 nucleotides which is not present in the cell containing the activated transcription factors.

Thus, there is no teaching or suggestion in Hibma et al. of a kit comprising a solid support comprising a DNA sequence which binds an activated transcription factor present in a cell and a spacer comprising a double-stranded nucleotide sequence of at least 50 nucleotides which is not present in said cell.

Also, neither Peterson et al. nor Hibma et al. describe an antibody specific for the activated form of a transcription factor. Both Peterson and Hibma relate to detection of binding between a transcription factor and its cognate DNA sequence. As previously submitted, Peterson et al. relates to assays for detecting drugs which act by interfering with the binding between a transcription factor and its cognate DNA sequence. Hibma merely discloses an antibody based assay for detecting transcription factors. However, as previously noted, many transcription factors bind to DNA in both their inactive and activated forms (see page 9 of the Amendment filed April 27, 2007. Applicants note that neither Peterson nor Hibma suggest specifically detecting the activated forms of transcription factors. In fact, since many transcription factors

bind DNA in both the inactive and activated forms, the methods of Peterson and Hibma would not be capable of discriminating between activated transcription factors and inactive transcription factors.

Kaltschmidt et al. describe a monoclonal antibody that selectively recognizes the activated form of the p65 subunit of the NF-kB transcription factor in cultured cells. There is no teaching or suggestion in Kaltschmidt et al. for a kit comprising a solid support comprising a DNA sequence which binds an activated transcription factor present in a cell and a spacer comprising a double-stranded nucleotide sequence of at least 50 nucleotides which is not present in said cell.

In view of the foregoing, Applicants maintain that the cited references fail to support a *prima facie* case of obviousness. These references fail because they do not provide the requisite reasonable expectation of success, or teach all the limitations of the claimed invention. Because of these deficiencies, Applicants submit that the PTO has failed to articulate a *prima facie* case of obviousness, and as such, the present rejection of Claims 27-29, 31-33 and 36-38 under 35 U.S.C. 103(a) should be withdrawn.

The Examiner has rejected Claims 27, 30 and 35 under 35 USC §103(a) as being allegedly unpatentable over Peterson et al. (US 5,563,036), in view of Hibma et al. (1994 *Nucl. Acids Res.* **22**:3806-3807), and further in view of Kaltschmidt et al. (*Biol. Chem. Hoppe-Seyler* **376**:9-16; 1995), as applied to claims 27-29, 31-33 and 36-38 above, in view of Church et al. (US 6,326,489). Specifically, the Examiner stated that a person of ordinary skill in the art would have been motivated to combine the high throughput screening method of Church et al. with the transcription factor binding method of Peterson et al. to quantify transcription factor binding and screen compounds directed to double-stranded oligonucleotides immobilized on a solid surface.

As discussed above, the references of Peterson, Hibma and Kaltschmidt do not teach all of the claim limitations of a kit comprising a solid support comprising a DNA sequence which binds an activated transcription factor present in a cell and a spacer comprising a double-stranded nucleotide sequence of at least 50 nucleotides which is not present in said cell. In the context of microarrays, it is very important that the spacer sequence does not belong to the genome of the cells being assayed to avoid binding of the assayed, but more importantly of other transcription factors to the spacer sequence.

Church et al. do not cure these deficiencies of the combination of the primary references. Therefore, Claims 27, 30 and 35 are non-obvious over the cited art, and their rejection under 35 USC §103(a) should be withdrawn.

CONCLUSION

Applicants have endeavored to address all of the Examiner's concerns as expressed in the outstanding Office Action. Accordingly, amendments to the claims, the reasons therefor, and arguments in support of the patentability of the pending claim set are presented above. In light of the above amendments and remarks, reconsideration and withdrawal of the outstanding rejections is specifically requested. If the Examiner finds any remaining impediment to the prompt allowance of these claims that could be clarified with a telephone conference, the Examiner is respectfully requested to initiate the same with the undersigned.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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